

# UC Irvine

## UC Irvine Previously Published Works

### Title

In situ hybridization for c-fos mRNA reveals the involvement of the superior colliculus in the propagation of seizure activity in genetically epilepsy-prone rats.

### Permalink

<https://escholarship.org/uc/item/71s0q0gs>

### Journal

Epilepsy research, 26(3)

### ISSN

0920-1211

### Authors

Ribak, CE  
Manio, AL  
Navetta, MS  
et al.

### Publication Date

1997-03-01

### DOI

10.1016/s0920-1211(96)01011-x

### Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

# In situ hybridization for *c-fos* mRNA reveals the involvement of the superior colliculus in the propagation of seizure activity in genetically epilepsy-prone rats

Charles E. Ribak\*, Allan L. Manio, Marco S. Navetta, Christine M. Gall

*Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717, USA*

Received 5 June 1996; accepted 17 September 1996

## Abstract

Previous work showed that bilateral lesions made between the inferior and superior colliculi reduced the severity of audiogenic seizures in genetically epilepsy-prone rats (GEPR-9s), and indicated that the connections between these two structures are vital for the propagation of seizure activity. To determine the involvement of the superior colliculus (SC) in seizure propagation, GEPR-9s were given four audiogenic seizures within 1 h by ringing a loud bell, and their brains were processed 30 min later for in situ hybridization for *c-fos* mRNA. Brain sections from such rats showed dense labeling in both the dorsal cortex and external nucleus of the inferior colliculus. Labeling continued rostrally into the intermediate and deep layers of the SC and the periaqueductal gray region. In addition, other brain regions such as the amygdala, piriform cortex and dorsal endopiriform nucleus showed dense labeling for *c-fos* mRNA. Comparable increases were not observed in the brains of Sprague-Dawley (SD) rats receiving auditory stimulation or in unstimulated GEPR-9s and SD rats, thereby indicating that increases in stimulated GEPR-9s are seizure-specific. This study provides further evidence that the SC is involved in the propagation of seizure activity in GEPR-9s, and also demonstrates the activation of other brain regions by audiogenic seizures. © 1997 Elsevier Science B.V.

**Keywords:** Inferior colliculus; External nucleus; Dorsal cortex; Periaqueductal gray; Amygdala; Piriform cortex

## 1. Introduction

Previous studies have shown that audiogenic seizures can be initiated from a brainstem area

responsible for the processing of auditory input, the inferior colliculus (IC) [13,24,26,27]. For example, the injection of glutamate agonists or  $\gamma$ -aminobutyric acid (GABA) antagonists into the central nucleus of the IC (CN) in Sprague-Dawley (SD) rats causes audiogenic seizures [26]. Genetically epilepsy-prone rats (GEPRs) are a well es-

\* Corresponding author. Tel.: +1 714 8245494; fax: +1 714 8248549; e-mail: ceribak@uci.edu

tablished genetic model of audiogenic seizures and are derived from SD rats. The seizures in GEPR-9s, the GEPRs with severe audiogenic seizures, are caused by a loud, wide-range sound stimulus and are characterized by a wild running phase followed by a tonic-clonic phase [8]. Several studies have indicated that the CN is the site for seizure initiation in GEPRs [13,27]. In addition, abnormalities with both the GABAergic and glutamate systems exist in the CN [32–34,36]. Recently, two other regions of the IC, the dorsal cortex (DC) and external nucleus (EN), have been shown to be involved in the propagation of seizure activity in GEPRs [35].

The propagation of seizure activity from the IC in GEPRs involves activation of motor neurons in the reticular pontine oralis nucleus (RPO) that give rise to the reticulospinal pathway [1]. Recently, we revealed some structures that are important for the propagation of seizure activity from the CN to the RPO [35]. Specifically, bilateral lesions in the coronal plane between the CN and the EN blocked seizure activity in GEPR-9s. Intercollicular lesions between the IC and the superior colliculus (SC) attenuated seizure activity. Together with previous connectional data, these results led to the conclusion that the EN and deep layers of the SC were important for the spread of seizure activity between the IC and RPO. A recent preliminary study provided further data to support a role for the deep layers of the SC in seizure propagation in GEPR-3s, the moderate seizure strain [28].

Expression of the proto-oncogene *c-fos* is a useful marker for elevated levels of neuronal activity generated in the brain when it is subjected to different types of stimuli. This marker has been linked to long term changes in the brain and is thought to be a key messenger in mediating these changes [29]. The mapping of *c-fos* expression has been used to study several models of epilepsy [5,7,9,11,15,19–23,25,38,43]. Its protein, Fos, has been shown to increase in the IC, lateral lemniscus and medial geniculate nucleus (MGB) following kindled auditory seizures and also to increase in some limbic structures depending upon the number of seizures elicited and their severity [40]. In GEPR-9s that experienced a single audiogenic

seizure, the periaqueductal gray area showed Fos immunoreactivity but the SC did not [4]. These two studies did not demonstrate a role for the SC in the circuitry for seizure propagation in a genetic model of audiogenic seizures. However, another study showed increased Fos protein immunoreactivity in the deep layers of the SC in kindled Wistar rats [41].

To identify the brain regions involved in seizure propagation in GEPR-9s, we used in situ hybridization to examine levels of *c-fos* mRNA following four audiogenic seizures. Densitometric analysis was performed to quantify regional changes in labeling. Particular attention was given to the SC and subnuclei of the IC because they were proposed to be involved in the circuitry for seizure propagation in GEPR-9s [35]. In situ hybridization for *c-fos* mRNA was used in this study rather than immunoreactivity for the Fos protein because it is likely to provide a more sensitive method to detect the expression of this immediate early gene [9]. The results of the present study show that the intermediate and deep layers of the SC express *c-fos* mRNA at elevated levels following seizures in GEPR-9s, and provide further support that the deep layers of the SC are involved in the propagation of audiogenic seizures.

## 2. Materials and methods

### 2.1. Animals and treatment

This study utilized 11 rats, seven GEPR-9s and four SD rats. The GEPR-9s were scored for their seizure severity when exposed to an auditory stimulus using the schedule outlined by Dailey et al. [8]. Five of the GEPR-9s and two SD rats were subjected to four stimuli in a 1 h period. The intensity of the sound produced was 106 dB on the SPL rating scale, A-weighted. The duration of the stimulus was about 3 s, and this time period was sufficient for the initiation of seizures in GEPR-9s. The duration of the stimulus for the two SD stimulated controls was approximately the same. Two GEPR-9s and two SD rats were not subjected to the audiogenic stimulation; these ‘unstimulated’ controls were processed for in situ

hybridization in parallel with tissue from experimental rats. Rats were given an overdose of sodium pentobarbital, 30 min after the final auditory stimulus. This survival period was chosen to optimize the increase in *c-fos* mRNA elicited by stimulation [30,39]. All rats were intracardially perfused with a 0.9% saline in 0.1 M phosphate buffer (PB) followed by 4% paraformaldehyde in PB (pH 7.4). The brains were placed in the fixative at 4°C for 48–72 h, and then cryoprotected in a 20% sucrose and 4% paraformaldehyde/PB solution. The brains were sectioned in the sagittal plane at a thickness of 30  $\mu$ m using a freezing microtome. The sections were collected into cold 4% paraformaldehyde/PB, where they were stored at 4°C until they were ready to be processed for in situ hybridization.

## 2.2. In situ hybridization

Sections were processed for in situ hybridization to determine the levels of *c-fos* mRNA expression using a  $^{35}$ S-labeled RNA probe complementary to rat *c-fos* mRNA (i.e. to positions 583–1250 of clone pc-*fes* rat-1 by Curran et al. [6]). The anti-sense cRNA was transcribed from *Pst*I-linearized recombinant clone pBS/rfos with T7 RNA polymerase in the presence of  $^{35}$ S-labeled uridine 5'-(alpha thio)-triphosphate. The sense RNA sequence was generated from the same template using T3 RNA polymerase after linearization with *Eco*RI.

In situ hybridization was carried out according to the methods outlined in Gall et al. [18]. Briefly, the sections were washed in 0.1 M glycine, treated with proteinase K (1  $\mu$ g/ml in 0.1 M Tris buffer, pH 8.0, with 50 mM EDTA) at 37°C for 30 min, rinsed in 0.25% acetic anhydride in 0.1 M triethanolamine, washed twice with  $2 \times$  saline sodium citrate (SSC) buffer (pH 7.0) and incubated in the hybridization buffer. The hybridization buffer contained 50% formamide, 10% dextran sulfate, 7 mg/ml bovine serum albumin, 0.7% ficoll, 0.7% polyvinyl pyrrolidone, 0.15 mg yeast tRNA, 0.33 mg/ml denatured herring sperm DNA and 40  $\mu$ M dithiothreitol. The hybridization incubation was performed at 60°C for 40–48 h with the cRNA at a concentration of  $1 \times 10^6$

cpm/100  $\mu$ l. Following hybridization, the sections were treated with ribonuclease A (20  $\mu$ g/ml in 10 mM Tris–saline, pH 8.0, with 1 mM EDTA) for 30 min at 45°C, and washed through decreasing concentrations of SSC to a final wash in  $0.1 \times$  SSC at 60°C. The tissue was then mounted onto gelatin-coated slides and left to air dry.

After drying, the slides were placed on Amersham B-max film and exposed for 3 days. This film was used for densitometric analysis. Slides were defatted and processed for emulsion autoradiography (Kodak NTB-2 mixed 1:1 with H<sub>2</sub>O) with an exposure interval of 4 weeks at 4°C. After emulsion development (Kodak D19 mixed 1:1 with H<sub>2</sub>O), the tissue was stained with cresyl violet and coverslipped with Permount.

## 2.3. Densitometric analysis

A microcomputer imaging device system ((MCID) Image Research, St. Catherine's, Ontario, Canada) was used for the densitometric analysis of the film autoradiograms. Sections of radiolabeled brain paste standards were exposed to the film along with the tissue [17]. These standards were used to calibrate density measures from the 18 brain regions evaluated in GEPR-9s and SD rats (Table 1), and to verify that tissue labeling densities were in the linear portion of the calibration curve. Labeling density measures are expressed as cpm/25  $\mu$ g of protein according to the rating of the brain paste standards. The examined brain regions were identified in the sagittal plane using a standard rat stereotaxic atlas [31]. At least three tissue sections were measured for each region from each rat.

## 3. Results

The in situ hybridization preparations obtained from stimulated GEPR-9s showed specific hybridization for  $^{35}$ S-labeled *c-fos* cRNA after four audiogenic seizures. Both the X-ray film and emulsion autoradiograms had many brain regions with dense labeling, and these regions coincided well between the two types of preparations. The densitometric analysis was made using the X-ray film.

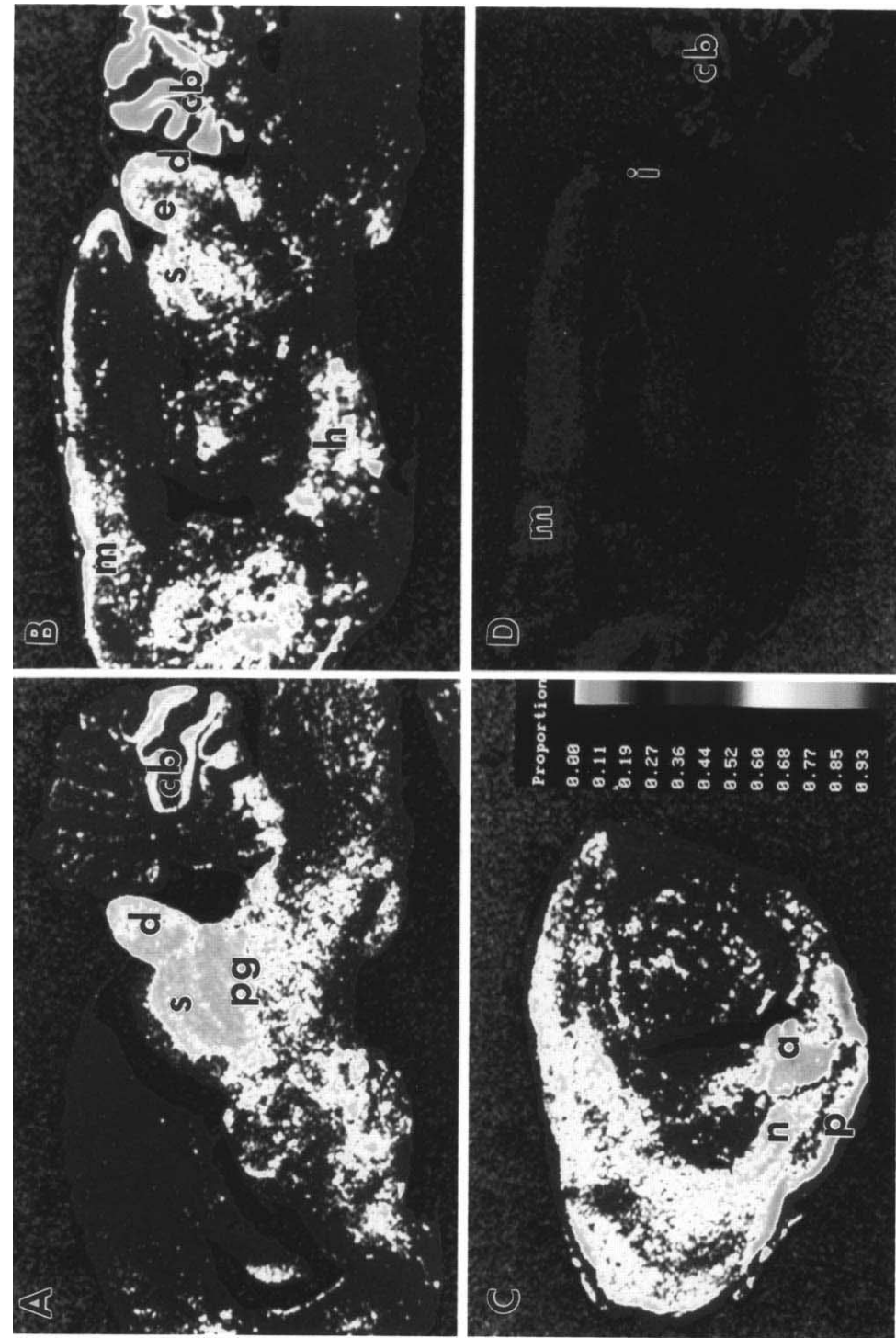


Fig. 1. Calibrated pseudocolor images of film autoradiograms showing the in situ hybridization to *c-fos* mRNA in parasagittal sections. (A–C) Sections obtained from a stimulated GEPR-9 following four seizures in 1 h whereas (D) is from an ‘unstimulated’ GEPR-9. In (A), the most medial of the three sections, the greatest labeling (red areas) occurs in the dorsal cortex of the IC (d), intermediate and deep layers of the SC (s), periaqueductal gray (pg) and folia of the cerebellum (cb). In (B), dense midbrain labeling continues from the dorsal cortex (d) to the external nucleus (e) of the IC and rostrally into the deep layers of the SC (s). In this plane, the hypothalamus (h), motor cortex (m) and folia of the cerebellum (cb) are also greatly labeled. In (C), the most lateral of the three sections, three limbic structures are greatly labeled, the amygdala (a), piriform cortex (p) and dorsal endopiriform nucleus (n). In (D), only light labeling is found in the IC (i), cerebellum (cb) and motor cortex (m) (magnification  $\times 10$ ).

### 3.1. Qualitative observations

Parasagittal sections ranging from 0.4 to 4.2 mm from the midline were evaluated for levels of *c-fos* mRNA expression. This description is based upon the data from five GEPR-9s that received audiogenic stimulation and had four seizures in

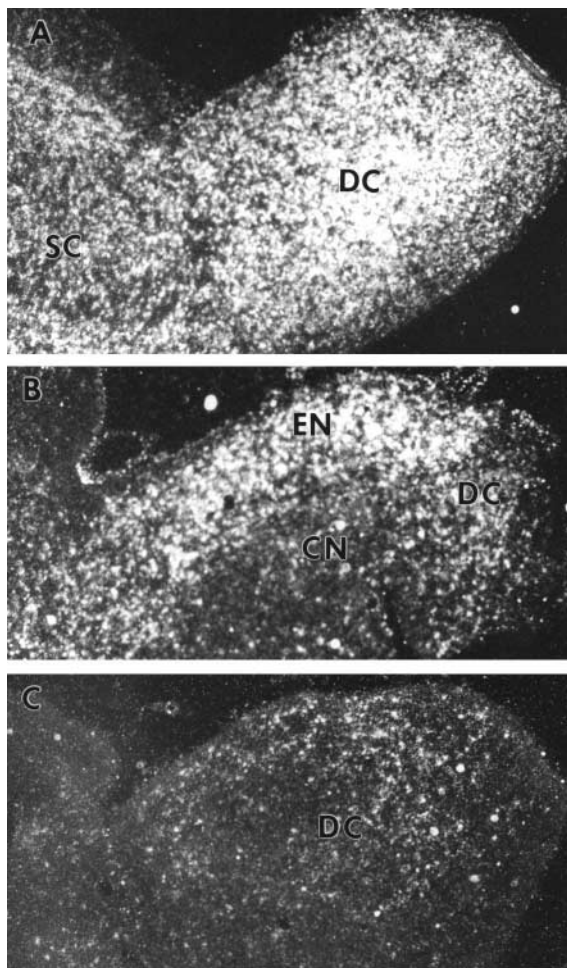


Fig. 2. Darkfield photomicrographs from tissue autoradiograms of parasagittal sections show in situ hybridization to *c-fos* mRNA in the midbrain of a stimulated GEPR-9 (A and B) and a stimulated SD rat (C). (A) shows numerous *c-fos* labeled cells in the DC of the IC that appear to be continuous with the labeled neurons in the intermediate and deep layers of the SC (SC). (B) shows the three main subdivisions of the IC in a more lateral section. Note the greater number of labeled neurons in the EN and DC of the IC than in the CN. (C) shows only a few scattered labeled neurons in the DC (magnification  $\times 60$ ).

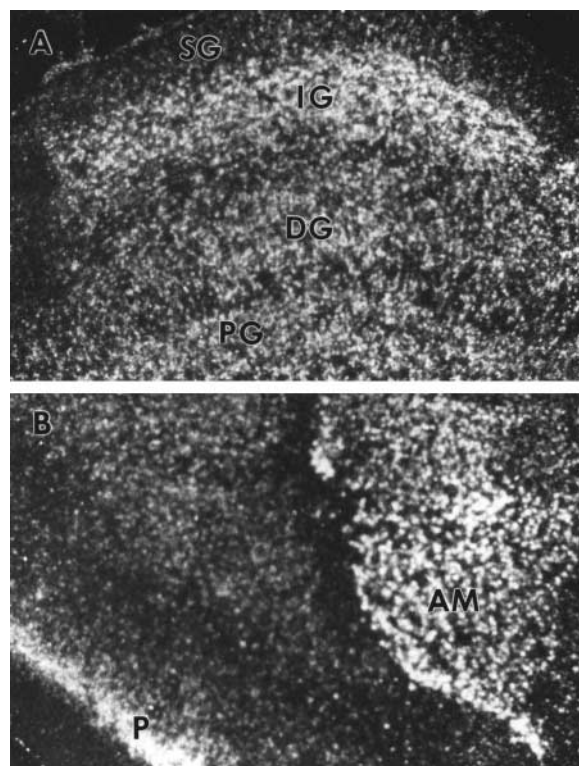


Fig. 3. Darkfield photomicrographs of autoradiograms of parasagittal sections to show in situ hybridization to *c-fos* mRNA from a stimulated GEPR-9. (A) shows numerous labeled neurons in the intermediate (IG) and deep (DG) layers of the SC and the subjacent periaqueductal gray (PG). Note that the superficial layer (SG) of the SC has only a few labeled neurons. (B) was obtained from a section that was 4.2 mm lateral to midline to show the labeling of the piriform cortex (P) and the amygdala (AM) (magnification  $\times 60$ ).

1 h. The most medial sections of the brainstem revealed dense labeling for *c-fos* mRNA in the DC of the IC (Fig. 1A and 2A), intermediate and deep layers of the SC (Fig. 1A and Fig. 3A) and the periaqueductal gray (Fig. 1A and 3A). At 1.4 mm lateral from the midline, the DC, and the intermediate and deep layers of the SC continued to show dense labeling. Other areas that displayed labeling in these sections included several folia in the cerebellum, the thalamus, and the septum. More lateral sections, 2.4 mm from the midline, revealed dense labeling of cells in the DC and EN of the IC whereas the CN of the IC was only lightly labeled (Figs. 1B and 2B). Once again, the dense labeling continued rostrally into the inter-

mediate and deep layers of the SC (Fig. 1B). The motor cortex and several cerebellar folia were also densely labeled in these sections (Fig. 1A). Sections analyzed at 4.2 mm lateral from the midline showed dense labeling in the amygdala, dorsal endopiriform nucleus, and piriform cortex (Fig. 1C and 3B).

SD rats that were stimulated by the same frequency and for the same length of time as the stimulated GEPR-9s did not show dense *c-fos* mRNA expression. Some scattered labeled cells were found in the DC (Fig. 2C), motor cortex and cerebellum. Labeling in the EN and CN of these rats was much less than that in stimulated GEPR-9s.

The two other groups of rats that were examined included SD rats and GEPR-9s that were not subjected to any audiogenic stimulation. Brain sections from these ‘unstimulated’ rats showed a similar distribution of *c-fos* mRNA expression in that they both showed only a few labeled neurons scattered throughout the brain (Fig. 1D).

Table 1  
Densitometric data for many brain regions following in situ hybridization to *c-fos* mRNA

Structures	Stimulated GEPR-9	Unstimulated GEPR-9	Stimulated SD	Unstimulated SD
Brainstem Structures				
External nucleus, IC	1289.8 ± 191.2	123.3 ± 29.4	228.7 ± 16.5	229.2 ± 3.7
Dorsal cortex, IC	1333.2 ± 215.6	119.5 ± 17.6	207.2 ± 2.4	226.0 ± 22.2
Central nucleus, IC	990.7 ± 182.9	142.3 ± 32.1	221.0 ± 9.1	216.0 ± 22.2
Superficial gray, SC	699.8 ± 123.0	137.6 ± 17.6	205.3 ± 24.1	184.8 ± 17.1
Intermediate gray, SC	1490.6 ± 31.0	119.0 ± 12.1	247.2 ± 34.1	217.3 ± 6.9
Deep gray, SC	1176.7 ± 183.0	110.1 ± 6.0	229.7 ± 29.6	203.0 ± 12.4
Periaqueductal gray	1383.8 ± 241.0	115.9 ± 8.5	213.3 ± 3.4	183.7 ± 24.1
Reticularis pont. oralis	558.9 ± 69.2	123.2 ± 12.6	184.3 ± 9.6	183.4 ± 5.5
Parvicellular retic. n.	412.0 ± 59.9	106.3 ± 16.3	174.6 ± 20.9	168.8 ± 1.0
Deep mesencephal. n.	770.0 ± 94.4	128.4 ± 8.2	195.2 ± 5.1	213.6 ± 2.5
Telencephalic structures				
Motor cortex	894.7 ± 171.3	248.7 ± 12.6	524.7 ± 21.7	419.1 ± 12.5
Piriform cortex	1769.3 ± 334.8	292.3 ± 58.8	894.1 ± 1.8	435.4 ± 90.6
Amygdala	1448.8 ± 175.8	239.1 ± 39.1	455.1 ± 30.4	251.0 ± 1.8
Dorsal endopiriform n.	1515.5 ± 293.7	269.6 ± 42.1	575.7 ± 4.4	322.6 ± 40.3
Hippocampus	348.2 ± 84.0	118.0 ± 20.7	184.7 ± 20.0	186.7 ± 20.0
Diencephalic structures				
Thalamus	697.0 ± 119.2	152.6 ± 8.3	278.0 ± 9.8	231.2 ± 0.3
Hypothalamus	845.6 ± 100.0	138.1 ± 5.3	217.3 ± 9.0	193.9 ± 4.6
Medial geniculate n.	958.5 ± 254.4	133.1 ± 4.1	267.7 ± 12.7	240.1 ± 24.9

The values are mean ± S.E.

3.2. Quantitative observations

The densities of labeling within specific brain regions were analyzed quantitatively using calibrated densitometric measures of the film autoradiograms. The EN, DC, intermediate and deep layers of the SC, and periaqueductal gray in stimulated GEPR-9s had mean hybridization values for *c-fos* mRNA that were in the 1100–1500 cpm/25 μg range. These values were more than ten times the values for the same brain regions from ‘unstimulated’ GEPR-9s (Table 1). Although other brain areas had mean values that were above 1000 cpm/25 μg, the values for these areas were not increased to as great an extent above measures found in ‘unstimulated’ GEPR-9s. In particular, in the piriform cortex, amygdala and the dorsal endopiriform nucleus (Table 1), the labeling densities were 5.5–7.1 times greater than that in ‘unstimulated’ GEPR-9s. Similarly, in the CN, motor cortex, medial geniculate nucleus, hypothalamus and deep mesencephalic nucleus la-

beling densities were five to seven times greater than those of ‘unstimulated’ GEPR-9s. Other examined brain regions showed elevated hybridization densities in stimulated GEPR-9s as compared to control rats; the smallest increase was found in the hippocampus where labeling was only three times greater than the measures from ‘unstimulated’ GEPR-9s.

The three groups of control rats, the ‘stimulated’ and ‘unstimulated’ SD rats and the ‘unstimulated’ GEPR-9s, showed similar labeling densities that were mainly between 100 and 300 cpm/25  $\mu$ g for all regions. Measures from the two SD rat groups were comparable whereas the ‘unstimulated’ GEPR-9s displayed slightly lower values in all brain regions measured (Table 1). In particular, values were much lower in the motor cortex, piriform cortex, amygdala and dorsal endopiriform nucleus of ‘unstimulated’ GEPR-9s as compared to the two SD rat groups.

#### 4. Discussion

This study used in situ hybridization to detect *c-fos* mRNA in GEPR-9s following four seizures in 1 h. The major finding of this study is that neurons in the intermediate and deep layers of the SC in stimulated GEPR-9s express a much greater amount of *c-fos* mRNA than those in the ‘unstimulated’ GEPR-9s (i.e. greater than ten times more). In addition, the pericentral region of the IC (the EN and DC), periaqueductal gray, amygdala, piriform cortex and dorsal endopiriform nucleus were shown to express *c-fos* mRNA at high levels in stimulated GEPR-9s.

An earlier study found no increase in Fos immunoreactivity after a single seizure within the SC, although the adjacent periaqueductal gray area was intensely labeled [4]. In contrast, the present study showed increased *c-fos* mRNA levels in the SC following four seizures. A previous methodological study has shown that in situ hybridization provides a greater sensitivity for detecting changes in *c-fos* gene expression than immunocytochemistry [9]. This may be due, in part, to the masking effect of immunostaining that is unrelated to the ‘test’ stimulus. Fos and

Fos-related antigens may be elevated within scattered neurons due to the effects of stress, hormones, or neuronal activity occurring within a period of hours preceding the test interval [30]. These proteins are detected by most Fos antibodies and can confound the interpretation of immunocytochemical results. An analysis of *c-fos* mRNA expression circumvents some of these problems of specificity, and limits the observations to recent transcriptional events due to the very rapid turnover of this mRNA species.

Three controls were used to determine if significant amounts of *c-fos* mRNA are present without auditory stimulation and the extent to which auditory stimulation can induce *c-fos* mRNA expression in the absence of seizures. In ‘unstimulated’ GEPR-9s and SD rats, *c-fos* mRNA levels were low, as they were in SD rats that experienced audiogenic stimulation (Table 1). These findings show that *c-fos* mRNA is not expressed in significant amounts in untreated GEPR-9s and SD rats and that audiogenic stimulation alone is not sufficient to increase significantly *c-fos* mRNA labeling in the SC and other brain regions that were greatly labeled in stimulated GEPR-9s. Thus, these data are consistent with the results of previous work which showed that audiogenic seizures induce *c-fos* mRNA expression in several brain regions of kindled rats [40,41]. It should be noted that the ‘unstimulated’ GEPR-9s had somewhat lower values than both types of SD rats. The reason for this finding may relate to the unique GEPR-9 genetic composition.

The CN is considered to be the site of initiation of audiogenic seizures in GEPR-9s [32]. GABA is a major inhibitory neurotransmitter in the CN [14], and GEPR-9s display increased numbers of immunocytochemically-labeled GABAergic neurons in the CN [36]. A recent in situ hybridization study confirmed this finding by showing increased numbers of labeled neurons that express *GAD<sub>67</sub>* mRNA in the IC of GEPR-9s [34]. An additional finding from this study was that the superficial layers of the SC expressed a high density of *GAD<sub>67</sub>* mRNA in GEPR-9s indicating that GABA is synthesized in greater amounts in the SC of GEPR-9s, which may be a result of audio-



genic seizures. This observation was interpreted as further evidence for the inclusion of the SC in the pathway of seizure propagation in these rats because the increased GABA expression may counteract the increased excitability from seizures. It is interesting to note that the site of initiation of audiogenic seizures in GEPR-9s, the CN, does not display as many *c-fos* mRNA positive neurons as do the pericentral nuclei of the IC, the EN and DC, and the intermediate and deep layers of the SC following audiogenic stimulation (see Table 1). Since these densely-labeled structures appear to play a role in the route of seizure propagation through the brain (see below), this result suggests that the presumed site of seizure initiation, the CN, is not activated as greatly as the brain regions involved in seizure propagation.

The brainstem regions containing the highest levels of *c-fos* mRNA in stimulated GEPR-9s were the pericentral region of the IC, the intermediate and deep layers of the SC and the periaqueductal gray; hybridization in each of these regions was more than ten-fold greater than in 'unstimulated' GEPR-9s. Most of these structures were proposed to be involved in the circuitry for seizure propagation in GEPR-9s based on a study that examined the effects of midbrain lesions on seizure activity [35]. Thus, it was hypothesized that following the initiation of seizures in the CN, the signal would be sent to the EN and DC. From these two nuclei, the signal would be passed to the intermediate and deep layers of the SC which are known to receive bilateral projections from the EN and DC [2]. The propagation of seizure activity would continue by an ipsilateral projection from the deep layers of the SC to the RPO [37,42], and from here to the motor neurons of the spinal cord via the reticulospinal tract. Our data also showed that clusters of cells in the RPO were labeled for *c-fos* mRNA in stimulated GEPR-9s. However, it is difficult to determine whether such clusters are involved in seizure propagation because the RPO was not labeled throughout its full extent. Therefore, most of the major structures proposed to be involved in seizure propagation in GEPR-9s were greatly labeled in the present study, including the pericentral region of the IC and the intermediate and deep layers of the SC.

The dense labeling of *c-fos* mRNA in the periaqueductal gray area suggests that it may also be involved in the propagation of seizures, however, its role in this regard is unclear. This finding was consistent with the dense Fos immunoreactivity in this structure in stimulated GEPR-9s [4]. Based on the connectional data, it is difficult to explain how the periaqueductal gray may play a role in the propagation of seizure activity because it lacks direct projections to the spinal cord. Instead, this region may become activated as a result of the seizures and may influence the reticular formation through its extensive projections [3].

Previous studies have proposed a seizure pathway in the GEPR-9 model that involves the propagation of activity from the IC to the MGB and then to the RPO [12]. This alternative pathway for the propagation of seizures in GEPR-9s is not expected to play a significant role based on the current findings; *c-fos* mRNA levels were much lower in the MGB than in the SC in our material. Thus, the major route for the propagation of seizures probably involves the IC projection to the SC.

Additional structures that exhibited elevated *c-fos* mRNA expression in stimulated GEPR-9s included the amygdala, piriform cortex and dorsal endopiriform nucleus. Evidence of dense labeling in these brain regions supports previous work showing that kindled seizures increase neuronal activity in forebrain structures. Forebrain *c-fos* expression in these structures has been suggested to indicate long term neuronal changes rather than actual involvement in the route of seizure propagation [40]. In regard to this, it is worth noting that *c-fos* mRNA is modestly elevated in these structures in stimulated SD rats as compared to 'unstimulated' SD rats. Finally, it is interesting that another limbic structure, the hippocampus, was not labeled as greatly as these limbic structures following audiogenic seizures in GEPR-9s, although it does express high levels of *c-fos* mRNA and Fos protein in other models of epilepsy [10,16,20,29]. This reinforces the idea that labeling patterns are specific for particular seizure paradigms and relate to the brain regions that are involved with the initiation and propagation of seizure activity, instead of a general response to seizures.

The dense labeling of the motor cortex and cerebellum in stimulated GEPR-9s was probably due to the intense movement associated with the audiogenic seizures. In control rats that remained relatively quiescent during this period, the labeling in these structures was minimal.

## Acknowledgements

The authors gratefully acknowledge the critical reading of the manuscript by Dr. Ursula Hess and the technical support provided by Yashoda Jhurani, Jeff Ingeman, Curtis Stinnis, Stephen Gold, Julie Wong, and Wendy Gaza. This research was supported by NIH grants NS-15669 (CER) and NS-26748 (CMG).

## References

- [1] Browning, R.A., Neuroanatomical localization of structures responsible for seizures in the GEPR: Lesion studies, *Life Sci.*, 39 (1986) 857–867.
- [2] Cadusseau, J. and Roger, M., Afferent projections to the superior colliculus in the rat, with special attention to the deep layers, *J. Hirnforsch.*, 26 (1985) 667–681.
- [3] Cameron, A.A., Kahn, I.A., Westlund, K.N. and Willis, W.D., The efferent projections of the periaqueductal gray in the rat: a *Phaseolus vulgaris*-leucoagglutinin study. II. Descending projections, *J. Comp. Neurol.*, 351 (1995) 585–601.
- [4] Chakravarty, D.N., Faingold, C.L., Terry, W.J. and Naritoku, D.K., Audiogenic seizures in the genetically epilepsy prone rat induce *c-fos* proto-oncogene expression in the inferior colliculus and periaqueductal gray, *Soc. Neurosci. Abstr.*, 19 (1993) 605.
- [5] Clark, M., Post, R.M., Weiss, S.R., Cain, C.J. and Nakajima, T., Regional expression of *c-fos* mRNA in rat brain during the evolution of amygdala kindled seizures, *Mol. Brain Res.*, 11 (1991) 55–64.
- [6] Curran, T., Gordon, M.B., Rubino, K.L. and Sambucetti, L.C., Isolation and characterization of the *c-fos*(rat) cDNA and analysis of post-translational modification in vitro, *Oncogene*, 2 (1987) 79–84.
- [7] Dave, J.R., Tabakoff, B. and Hoffman, P.L., Ethanol withdrawal seizures produce increased *c-fos* mRNA in mouse brain, *Mol. Pharmacol.*, 37 (1990) 367–371.
- [8] Dailey, J.W., Reigel, C.E., Mishra, P.K. and Jobe, P.C., Neurobiology of seizure predisposition in the genetically epilepsy-prone rat, *Epilepsy Res.*, 3 (1989) 3–17.
- [9] Dragunow, M. and Faull, R., The use of *c-fos* as a metabolic marker in neuronal pathway tracing, *J. Neurosci. Methods*, 29 (1989) 261–265.
- [10] Dragunow, M., Yamada, N., Bilkey, D.K. and Lawlor, P., Induction of immediate-early gene proteins in dentate granule cells and somatostatin interneurons after hippocampal seizures, *Mol. Brain Res.*, 13 (1992) 119–126.
- [11] Ebert, U. and Löscher, W., Strong induction of *c-fos* in the piriform cortex during focal seizures evoked from different limbic brain sites, *Brain Res.*, 671 (1995) 338–344.
- [12] Faingold, C.L. and Naritoku, D.K., *Drugs for Control of Epilepsy*, CRC Press, Florida, 1992, pp. 278–308.
- [13] Faingold, C.L., Millan, M.H., Boersma, C.A. and Meldrum, B.S., Excitant amino acids and audiogenic seizures in the genetically-epilepsy prone rat. I. Afferent seizure initiation pathway, *Exp. Neurol.*, 99 (1988) 678–686.
- [14] Faingold, C.L., Marcinczyk, M.J., Casebeer, D.J., Randall, M.E., Arneric, S.P. and Browning, R.A., GABA in the inferior colliculus plays a critical role in control of audiogenic seizures, *Brain Res.*, 640 (1994) 40–47.
- [15] Gall, C., Lauterborn, J., Isackson, P. and White, J., Seizures, neuropeptide regulation, and mRNA expression in the hippocampus, *Prog. Brain Res.*, 83 (1990) 371–390.
- [16] Gall, C., Murray, K. and Isackson, P.J., Kainic acid-induced seizures stimulate increased expression of nerve growth factor mRNA in rat hippocampus, *Mol. Brain Res.*, 9 (1991) 113–123.
- [17] Gall, C.M., Berschauer, B. and Isackson, P.J., Basic fibroblast growth factor mRNA is increased in forebrain neurons and glia following recurrent limbic seizures, *Mol. Brain Res.*, 21 (1994) 190–205.
- [18] Gall, C., Lauterborn, J.C. and Guthrie, K.M., In situ hybridization: A sensitive measure of activity dependent changes in neuronal gene expression. In: W.E. Stumpf and H.F. Solomon (Eds.), *In Vitro/in Vivo Autoradiography and Correlative Imaging*, Academic Press, San Diego, 1995, pp. 379–399.
- [19] Herdegen, T., Sandkuhler, J., Gass, P., Kiessling, M., Bravo, R. and Zimmermann, M., JUN, FOS, KROX, and CREB transcription factor proteins in the rat cortex: basal expression and induction by spreading depression and epileptic seizures, *J. Comp. Neurol.*, 333 (1993) 271–288.
- [20] Jensen, F.E., Firkusny, I.R. and Mower, G.D., Differences in *c-fos* immunoreactivity due to age and mode of seizure induction, *Molec. Brain Res.*, 17 (1993) 185–93.
- [21] Labiner, D.M., Butler, L.S., Cao, Z., Hosford, D.A., Shin, C. and McNamara, J.O., Induction of *c-fos* mRNA by kindled seizures: complex relationship with neuronal burst firing, *J. Neurosci.*, 13 (1993) 744–751.
- [22] Lanaud, P., Maggio, R., Gale, K. and Grayson, D.R., Temporal and spatial patterns of expression of *c-fos*, *zif/268*, *c-jun* and *jun-B* mRNAs in rat brain following seizures evoked focally from the deep prepiriform cortex, *Exp. Neurol.*, 119 (1993) 20–31.
- [23] Le Gal La Salle, G. and Naquet, R., Audiogenic seizures evoked in DBA/2 mice induce *c-fos* oncogene expression in subcortical auditory nuclei, *Brain Res.*, 518 (1990) 308–312.

- [24] Ludvig, N. and Moshé, S.L., Different behavioral and electrographic effects of acoustic stimulation and dibutyryl cyclic AMP injection into the inferior colliculus in normal and in genetically epilepsy-prone rats, *Epilepsy Res.*, 3 (1989) 185–190.
- [25] Maggio, R., Lanaud, P., Grayson, D.R. and Gale, K., Expression of *c-fos* mRNA following seizures evoked from an epileptogenic site in the deep prepiriform cortex: regional distribution in brain as shown by in situ hybridization, *Exp. Neurol.*, 119 (1993) 11–19.
- [26] Millan, M.H., Meldrum, B.S. and Faingold, C.L., Induction of audiogenic seizure susceptibility by focal infusion of excitant amino acids or bicuculline into the inferior colliculus of normal rats, *Exp. Neurol.*, 189 (1986) 634–639.
- [27] Millan, M.H., Meldrum, B.S., Boersma, C.A. and Faingold, C.L., Excitant amino acids and audiogenic seizures in the genetically epilepsy-prone rat. II. Efferent seizure propagating pathway, *Exp. Neurol.*, 99 (1988) 687–698.
- [28] Mishra, P.K., Deoskar, V.U., Dailey, J.W. and Jobe, P.C., Role of the superior colliculus region in brainstem seizure circuitry, *Soc. Neurosci. Abstr.*, 20 (1994) 404.
- [29] Morgan, J.I. and Curran, T., Stimulus-transcription coupling in neurons: role of cellular immediate-early genes, *Trends Neurosci.*, 12 (1989) 459–462.
- [30] Morgan, J.I. and Curran, T., Proto-oncogene transcription factors and epilepsy, *Trends Pharmacol. Sci.*, 12 (1991) 343–349.
- [31] Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic Press, Sydney, 1986.
- [32] Ribak, C.E. and Morin, C.L., The role of the inferior colliculus in a genetic model of audiogenic seizures, *Anat. Embryol.*, 191 (1995) 279–295.
- [33] Ribak, C.E., Ruiz, G.T., Byun, M.Y. and Reiffenstein, R.J., Increased levels of amino acid neurotransmitters in the inferior colliculus of the genetically epilepsy-prone rat, *Epilepsy Res.*, 2 (1988) 9–13.
- [34] Ribak, C.E., Lauterborn, J.C., Navetta, M.S. and Gall, C.M., The inferior colliculus of GEPRs contains greater numbers of cells that express glutamate decarboxylase (*GAD<sub>67</sub>*) mRNA, *Epilepsy Res.*, 14 (1993) 105–113.
- [35] Ribak, C.E., Khurana, V. and Lien, N.T., The effect of midbrain collicular knife cuts on audiogenic seizure severity in the genetically epilepsy-prone rat, *J. Brain Res.*, 35 (1994) 303–311.
- [36] Roberts, R.C., Ribak, C.E. and Oertel, W.H., Increased numbers of GABAergic neurons occur in the inferior colliculus of an audiogenic model of genetic epilepsy, *Brain Res.*, 361 (1985) 324–338.
- [37] Shammah-Lagnado, S.J., Negrao, N., Silva, B.A. and Ricardo, J.A., Afferent connections of the nuclei reticularis pontis oralis and caudalis: a horseradish peroxidase study in the rat, *Neuroscience*, 20 (1987) 961–989.
- [38] Shehab, S., Coffey, P., Dean, P. and Redgrave, P., Regional expression of Fos-like immunoreactivity following seizures induced by pentylenetetrazole and maximal electroshock, *Exp. Neurol.*, 118 (1992) 261–274.
- [39] Shin, C., McNamara, J.O., Morgan, J.I., Curran, T. and Cohen, D.R. Induction of *c-fos* mRNA expression by afterdischarge in hippocampus of naive and kindled rats, *J. Neurochem.*, 55 (1990) 1050–1055.
- [40] Simler, S., Hirsch, E., Danover, L., Motte, J., Vergnes, M. and Marescaux, C., *c-fos* expression after single and kindled seizures in Wistar rats, *Neurosci. Lett.*, 175 (1994) 58–62.
- [41] Snyder-Keller, A.M. and Pierson, M., Audiogenic seizures induce *c-fos* in a model of developmental epilepsy, *Neurosci. Lett.*, 135 (1992) 108–112.
- [42] Yasui, Y., Tsumori, T., Ando, A., Domoto, T., Kayahara, T. and Nakano, K., Descending projections from the superior colliculus to the reticular formation around the motor trigeminal nucleus and the parvocellular reticular formation of the medulla oblongata in the rat, *Brain Res.*, 656 (1994) 420–426.
- [43] Winston, S.M., Hayward, M.D., Nestler, E.J. and Duman, R.S., Chronic electroconvulsive seizures down-regulate expression of the immediate-early genes *c-fos* and *c-jun* in rat cerebral cortex, *J. Neurochem.*, 54 (1990) 1920–1925.